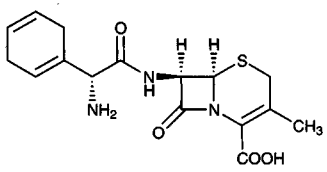
Cephradine



Molecular formula: $C_{16}H_{19}N_3O_4S$
Molecular weight: 349.41
CAS Registry No.: 38821-53-3, 58456-86-3 (dihydrate), 31828-50-9 (non-stoichiometric hydrate)
Merck Index: 2032
Lednicer No.: 2 440

SAMPLE

Matrix: blood
Sample preparation: 500 μL Plasma + 4 mL water + 3 mL 10% trichloroacetic acid, centrifuge at 800-1000 g for 5 min. Remove 3 mL of the supernatant and add it to 3 mL buffer, add 1 mL 0.6% hydrogen peroxide in buffer, heat in a boiling water bath for 55 min, cool to room temperature, add 1 mL prewarmed 1 M Na_2HPO_4, add 7 mL acetone: chloroform 40:60, shake vigorously for 5 min, centrifuge. Remove 5 mL of the organic layer and evaporate it to dryness under reduced pressure, reconstitute the residue in 100 μL MeOH containing IS, inject a 20 μL aliquot. (Prepare buffer by dissolving 21 g citric acid in 200 mL 1 M NaOH, make up to 1 L with water, adjust pH to 2.5 with 100 mM HCl.)

HPLC VARIABLES

Column: 250 × 4 5 μm Nucleosil C18
Mobile phase: MeOH:water 60:40
Column temperature: 55
Injection volume: 20
Detector: F ex 345 em 420

CHROMATOGRAM

Retention time: 6 (?)
Internal standard: methyl anthranylate (9 (?))
Limit of detection: 10 ng/mL

OTHER SUBSTANCES

Interfering: ampicillin, cephalexin

KEY WORDS

plasma; derivatization

REFERENCE

Miyazaki,K.; Ohtani,K.; Sunada,K.; Arita,T. Determination of ampicillin, amoxicillin, cephalexin, and cephradine in plasma by high-performance liquid chromatography using fluorometric detection, *J.Chromatogr.*, **1983**, *276*, 478–482.

SAMPLE

Matrix: blood
Sample preparation: 100 μL Serum + 10 μL 200 μg/mL cefadroxil in water + 100 μL 6% trichloroacetic acid, vortex, centrifuge at 9000 g for 10 min, inject 25 μL supernatant.

HPLC VARIABLES

Guard column: Waters Guard-Pak C18
Column: 200 × 4.6 5 μm Nucleosil SA
Mobile phase: 20 mM Ammonium dihydrogen phosphate to final concentration of 20 mM in water:MeOH:MeCN 30:35:35. The pH was adjusted to 3.0 with concentrated phosphoric acid.

Flow rate: 1.5
Injection volume: 25
Detector: UV 240

CHROMATOGRAM
Retention time: 10.5
Internal standard: cefadroxil
Limit of quantitation: 1000 ng/mL

KEY WORDS
serum

REFERENCE
Lindgren,K. Determination of cefadroxil in serum by high-performance liquid chromatography with cephradine as internal standard, *J.Chromatogr.*, **1987**, *413*, 347–350.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Serum + 10 μL 100 μg/mL cephradine in water + 100 μL MeCN, vortex, centrifuge at 9000 g for 10 min. Remove 100 μL supernatant, evaporate to dryness at room temperature under reduced pressure, dissolve residue in 100 μL 20 mM NaH_2PO_4 adjusted to pH 3.5 with phosphoric acid, centrifuge at 9000 g for 5 min, inject 20 μL supernatant.

HPLC VARIABLES
Guard column: Waters Guard Pak C18
Column: 200 × 4.6 5 μm Nucleosil C18
Mobile phase: MeCN:buffer 30:70, pH adjusted to 7.0 with NaOH (Buffer was 20 mM sodium phosphate and 5 mM tetrabutylammonium hydrogen sulfate.)
Flow rate: 1
Injection volume: 20
Detector: UV 265

CHROMATOGRAM
Retention time: 5
Internal standard: cefaclor
Limit of detection: 1000 ng/mL

KEY WORDS
serum

REFERENCE
Lindgren,K. Determination of cefaclor and cephradine in serum by ion-pair reversed-phase chromatography, *J.Chromatogr.*, **1987**, *413*, 351–354.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Serum + 10 μL 5 μg/mL cefixime in MeOH + 100 μL MeCN, vortex for 15 s, centrifuge at 14000 g for 2 min. Remove the supernatant and evaporate it under a stream of nitrogen, reconstitute in 100 μL mobile phase, inject a 50-80 μL aliquot.

HPLC VARIABLES
Guard column: RCSS Silica Guard Pak (Waters)
Column: 150 × 4.6 5μm Ultrasphere Octyl C8
Mobile phase: MeOH:12.5 mM pH 2.6 NaH_2PO_4 (pH adjusted with concentrated phosphoric acid) 20:80

Flow rate: 2
Injection volume: 50-80
Detector: UV 240

CHROMATOGRAM
Retention time: 21
Internal standard: cefixime (11)
Limit of detection: 1000 ng/mL

OTHER SUBSTANCES
Extracted: cefaclor, cefadroxil, cephalexin
Noninterfering: acetaminophen, cimetidine, diazepam, digoxin, ibuprofen, phenytoin, propranolol, salicylic acid, warfarin.

KEY WORDS
serum

REFERENCE
McAteer,J.A.; Hiltke,M.F.; Silber,B.M.; Faulkner,R.D. Liquid-chromatographic determination of five orally active cephalosporins -cefixime, cefaclor, cefadroxil, cephalexin, and cephradine -in human serum, *Clin.Chem.*, **1987**, *33*, 1788–1790.

SAMPLE
Matrix: blood, urine
Sample preparation: 1 mL Plasma + 1 mL 6% trichloroacetic acid, mix, centrifuge at 4000 rpm for 10 min, inject an aliquot of the supernatant. Inject an aliquot of urine directly.

HPLC VARIABLES
Guard column: 10 × 4 7 μm Lichrosorb RP 18
Column: 250 × 4 7 μm Lichrosorb RP 18
Mobile phase: MeCN:25 mM pH 7 phosphate buffer 10:90
Flow rate: 1
Injection volume: 10
Detector: F ex 385 em 485 following post-column reaction. The column effluent mixed with 200 μg/mL fluorescamine in MeCN pumped at 0.25 mL/min and the mixture flowed through a 4.5 m × 0.25 mm ID coil of PTFE tubing to the detector; UV 260

CHROMATOGRAM
Limit of detection: 1.8 ng/mL (F), 2.7 ng/mL (UV)

OTHER SUBSTANCES
Also analyzed: cefaclor, cefroxadine, cephalexin
Noninterfering: amidopyrin, aspirin, barbital, caffeine, cefmenoxime, cefotaxime, ceftizoxime, ceftriaxone, cetazidime, diazepam, dibekacin, gentamycin, kanamycin, lidocaine, netilmicin, tetracaine, theophylline, tobramycin

KEY WORDS
post-column reaction; plasma; F detection may be less susceptible to interferences

REFERENCE
Blanchin,M.D.; Fabre,H.; Mandrou,B. Fluorescamine post-column derivatization for the HPLC determination of cephalosporins in plasma and urine, *J.Liq.Chromatogr.*, **1988**, *11*, 2993–3010.

SAMPLE
Matrix: bulk, formulations
Sample preparation: Dissolve in water to a concentration of 40 μg/mL, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:water:acetic acid 30:70:0.1
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 13
Limit of quantitation: 1800 ng/mL

OTHER SUBSTANCES
Simultaneous: impurities, cefadroxil, cephapirin, ceftizoxime, cefaclor, cefotaxime, cephalexin, cefazolin, cefoxitin, cefoperazone, cefamandole, cephalothin, cefamandole nafate

REFERENCE
Ting,S. Reverse-phase liquid chromatographic analysis of cephalosporins, *J.Assoc.Off.Anal.Chem.*, **1988**, *71*, 1123–1130.

SAMPLE
Matrix: solution

HPLC VARIABLES
Column: 250 × 4.6 Inertsil ODS-2
Mobile phase: MeOH:10 mM pH 6 acetate buffer 20:80
Column temperature: 40
Flow rate: 1
Detector: UV 220

OTHER SUBSTANCES
Also analyzed: antipyrine, benzoic acid, mannitol, 3-O-methyl-D-glucose, L-lactic acid, phenylalanine

REFERENCE
Ogihara,T.; Tamai,I.; Tsuji,A. Application of fractal kinetics for carrier-mediated transport of drugs across intestinal epithelial membrane, *Pharm.Res.*, **1998**, *15*, 620–625.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak phenyl
Mobile phase: MeOH:10 mM phosphate buffer 27:73, pH 3.6
Column temperature: 27
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: 11

OTHER SUBSTANCES
Simultaneous: ampicillin, cephalexin, cefaclor, amoxicillin

REFERENCE
Huang,H.-S.; Wu,J.-R.; Chen,M.-L. Reversed-phase high-performance liquid chromatography of amphoteric β-lactam antibiotics: effects of columns, ion-pairing reagents and mobile phase pH on their retention times, *J.Chromatogr.*, **1991**, *564*, 195–203.

SAMPLE
Matrix: solutions
Sample preparation: Adjust pH to 3.0 with HCl, inject an aliquot.

HPLC VARIABLES
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:20 mM pH 5.0 NaH_2PO_4 13:87
Flow rate: 1.6
Detector: UV 262

KEY WORDS
buffers

REFERENCE
Yuasa,H.; Amidon,G.L.; Fleisher,D. Peptide carrier-mediated transport in intestinal brush border membrane vesicles of rats and rabbits: cephradine uptake and inhibition, *Pharm.Res.*, **1993**, *10*, 400–404.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 10 μL aliquot.

HPLC VARIABLES
Column: 220 × 4.6 Spheri 5 ODS-224
Mobile phase: 100 mM sodium dodecyl sulfate, pH 6.72
Flow rate: 1
Injection volume: 10
Detector: UV 260

CHROMATOGRAM
Retention time: 5.5

OTHER SUBSTANCES
Simultaneous: cefazolin, cephalothin, cephaloridine, cephalexin, 7-aminocephalorospanic acid, 7-aminodesacetoxycephalosporanic acid

REFERENCE
Garcia Pinto,C.; Pérez Pavón,J.L.; Moreno Cordero,B. Micellar liquid chromatography of zwitterions: Retention mechanism of cephalosporins, *Analyst*, **1995**, *120*, 53–62.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 125 × 4.6 Lichrospher 100 RP-18
Mobile phase: MeOH:2.5 mM pH 5.6 sodium phosphate buffer 18:80
Flow rate: 1
Injection volume: 20
Detector: UV 274

CHROMATOGRAM
Retention time: 6
Limit of detection: 60 nM

OTHER SUBSTANCES
Simultaneous: cefoperazone, cefoxitin, cefuroxime, ceftazidime, cephalexin

KEY WORDS
comparison with capillary electrophoresis

REFERENCE
Choi,O.-K.; Song,Y.-S. Determination of cefuroxim levels in human serum by micellar electrokinetic capillary chromatography with direct sample injection, *J.Pharm.Biomed.Anal.*, **1997**, *15*, 1265–1270.

SAMPLE
Matrix: tissue
Sample preparation: Muscle, fat. 10 g Minced tissue + 1 mL 10 mM pH 3.0 phosphate buffer, let stand for 30 min, add 19 mL 5% trichloroacetic acid, chill to 5°, homogenize (Virtis model 45), centrifuge at 1000 g for 5 min, filter (0.2 μm), inject 2 mL of the filtrate onto column A with mobile phase A, elute to waste with mobile phase A for 1.5 min then flush contents of column A onto column B with mobile phase B, elute column B with mobile phase B and monitor the effluent. Liver, kidney. 10 g Minced tissue + 1 mL 10 mM pH 3.0 phosphate buffer, let stand for 30 min, add 19 mL 5% trichloroacetic acid, chill to 5°, homogenize (Virtis model 45), centrifuge at 1000 g for 5 min, filter (0.2 μm). 10 mL Filtrate + 20 mL dichloromethane:isopropanol 95:5, stir for 2 min, centrifuge at 1000 g for 5 min. Discard the organic layer, add 250 μL concentrated ammonia solution to the aqueous layer, add 20 mL dichloromethane:isopropanol 95:5, stir for 2 min, centrifuge at 1000 g for 5 min. Discard the organic layer, restore the initial pH of the aqueous layer with concentrated HCl. Inject 2 mL of this solution onto column A with mobile phase A, elute to waste with mobile phase A for 1.5 min then flush contents of column A onto column B with mobile phase B, elute column B with mobile phase B and monitor the effluent.

HPLC VARIABLES
Column: A 25 × 4 25-40 μm LiChroprep RP 18; B 4 × 4 5 μm LiChrospher 100 CH-18 +250 × 4 5 μm LiChrospher 100 CH-18
Mobile phase: A MeOH:10 mM pH 3.0 phosphate buffer 15:85; B MeOH:10 mM pH 3.0 phosphate buffer 30:70 (Every 30 injections change column A and column B guard column, flush with 60 mL MeOH:water 30:70 and 30 mL MeOH.)
Flow rate: 1
Injection volume: 2000
Detector: UV 260

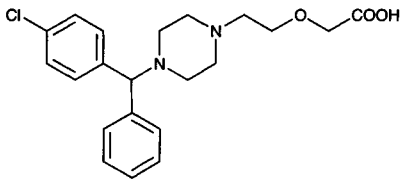
CHROMATOGRAM
Retention time: 15
Internal standard: cephradine

OTHER SUBSTANCES
Extracted: cephalexin

KEY WORDS
cow; muscle; fat; liver; kidney; cephradine is IS

REFERENCE
Leroy,P.; Decolin,D.; Nicolas,S.; Archimbault,P.; Nicolas,A. Residue determination of two coadministered antibacterial agents -cephalexin and colistin -in calf tissues using high-performance liquid chromatography and microbiological methods, *J.Pharm.Biomed.Anal.*, **1989**, *7*, 1837–1846.

Cetirizine



Molecular formula: $C_{21}H_{25}ClN_2O_3$
Molecular weight: 388.89
CAS Registry No.: 83881-51-0, 83881-52-1 (di HCl)
Merck Index: 2063
Lednicer No.: 4 118

SAMPLE

Matrix: blood
Sample preparation: 1 mL Serum + 50 μL 3 μg/mL IS + 1 mL 1 M pH 5.0 sodium citrate buffer + 3 mL ethyl acetate, vortex 1 min, centrifuge at 4000 rpm for 15 min, remove organic layer, repeat extraction. Combine organic layers and add 200 μL 1.7% phosphoric acid, vortex 1 min, centrifuge 5 min, remove and discard ethyl acetate layer, remove traces of ethyl acetate from aqueous layer using a stream of nitrogen, inject.

HPLC VARIABLES

Column: radial 4 μm NovoPak C18 radial compression
Mobile phase: MeCN:buffer 46:54 (Buffer was 10 mM pH 2.9 KH_2PO_4 + 20 mM sodium 1-decanesulfonate.)
Flow rate: 1.4
Detector: UV 229

CHROMATOGRAM

Retention time: 5.0
Internal standard: P-265, an ethoxy derivative of cetirizine (6.8)
Limit of detection: 2 ng/mL

OTHER SUBSTANCES

Simultaneous: hydroxyzine

KEY WORDS

serum

REFERENCE

Simons,K.J.; Watson,W.T.A.; Chen,X.Y.; Simons,F.E.R. Pharmacokinetic and pharmacodynamic studies of the H_1-receptor antagonist hydroxyzine in the elderly, *Clin.Pharmacol.Ther.*, **1989**, *45*, 9–14.

SAMPLE

Matrix: blood
Sample preparation: 200 μL Serum + 100 μL buffer + 100 μL 25% perchloric acid, vortex for 10 s, centrifuge at 2000 g for 3 min, inject a 200 μL aliquot. (Buffer was 50 mM $(NH_4)H_4PO_4$ containing 0.5 mL/L triethylamine, adjust pH to 2.5 with 85% phosphoric acid.)

HPLC VARIABLES

Guard column: 10 × 4.6 5 μm Hyperspheres ODS (Shandon)
Column: 250 × 4.6 Spherisorb S5 ODS2
Mobile phase: MeCN:MeOH:50 mM pH 2.5 ammonium phosphate 33:9:58
Column temperature: 35
Flow rate: 2
Injection volume: 200
Detector: UV 211

CHROMATOGRAM

Retention time: 6.4

Limit of detection: 20 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
serum

REFERENCE
Moncrieff,J. Determination of cetirizine in serum using reversed-phase high-performance liquid chromatography with ultraviolet spectrophotometric detection, *J.Chromatogr.*, **1992**, *583*, 128–130.

SAMPLE
Matrix: blood

Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPack C18

Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)

Column temperature: 30

Flow rate: 0.8

Injection volume: 50

Detector: UV 230

CHROMATOGRAM
Retention time: 5.59

Limit of detection: <120 ng/mL

KEY WORDS
whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifox-

amine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, tissue

Sample preparation: Blood. Dilute 1 mL plasma with 100 μL 1 M pH 5.0 phosphate buffer and 100 μL water, add 8 mL ethyl acetate, extract. Evaporate the organic layer, mix the residue with 500 μL 1.7% phosphoric acid and inject 200 μL aliquot of the lower layer. Tissue. Homogenize the brain with 2-fold the weight of water. Dilute 1500 μL brain homogenate with 500 μL 1 M pH 5.0 phosphate buffer, add 9 mL ethyl acetate, extract. Evaporate the organic layer, mix the residue with 400 μL 1.7% phosphoric acid, inject a 200 μL aliquot of the lower layer.

HPLC VARIABLES

Column: 150 × 4.6 Intersil PH
Mobile phase: MeCN:0.018% TFA 25:75
Flow rate: 0.7
Injection volume: 200
Detector: UV 230

CHROMATOGRAM

Limit of quantitation: 10 ng/mL (plasma), 15 ng/mL (brain)

KEY WORDS

brain; cat; mouse; pharmacokinetics; plasma; rat

REFERENCE

Kato,M.; Nishida,A.; Aga,Y.; Kita,J.; Kudo,Y.; Narita,H.; Endo,T. Pharmacokinetic and pharmacodynamic evaluation of central effect of the novel antiallergic agent betotastine besilate, *Arzneimittelforschung*, **1997**, *47*, 1116–1124.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using

a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 15.683

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM
Retention time: 8.89 (A), 5.29 (B)

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone,

methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
also details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE
Matrix: urine
Sample preparation: 500 μL Urine + 25 μL 100 μg/mL IS in water + 1 mL 1 M pH 5 citrate buffer + 6 mL chloroform, shake horizontally for 10 min, centrifuge at 3015 g at 4° for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100 μL mobile phase, inject a 10 μL aliquot.

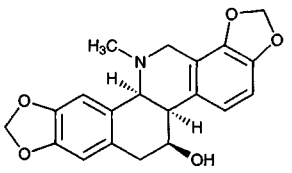
HPLC VARIABLES
Column: 250 × 4.6 Spherisorb 5ODS-2
Mobile phase: MeOH:THF:5 mM tetrabutylammonium phosphate (Pic A, Waters) 65:2:33
Flow rate: 1
Injection volume: 10
Detector: UV 230

CHROMATOGRAM
Retention time: 6.17
Internal standard: [2-[2-[4-(diphenylmethylene)-1-piperidinyl]ethoxy]ethoxy]-acetic acid (UCB J028) (5.53)
Limit of detection: 20 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

REFERENCE
Rosseel,M.T.; Lefebvre,R.A. Determination of cetirizine in human urine by high-performance liquid chromatography, *J.Chromatogr.*, **1991**, *565*, 504–510.

Chelidonine



Molecular formula: $C_{20}H_{19}NO_5$
Molecular weight: 353.37
CAS Registry No.: 476-32-4, 20267-87-2 ((±)-form), 88200-01-5 ((-)-form)
Merck Index: 2095

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 206.4

CHROMATOGRAM

Retention time: 3.625

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: solutions
Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 µg/mL, inject a 10 µL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 µm µBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 25:1.5:0.5:73
Flow rate: 1.5
Injection volume: 10
Detector: UV 254

CHROMATOGRAM

Retention time: 6

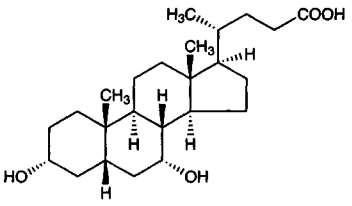
OTHER SUBSTANCES

Simultaneous: theobromine, theophylline, caffeine, 8-chlorotheophylline

REFERENCE

Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

Chenodiol



Molecular formula: $C_{24}H_{40}O_4$
Molecular weight: 392.58
CAS Registry No.: 474-25-9
Merck Index: 2096

SAMPLE

Matrix: bile

Sample preparation: 200 μL Bile + 4 M NaOH:MeOH 50:50, heat at 80° for 16 h, adjust pH to 1.5 with 6 M HCl, extract three times with 10 mL portions of ethyl acetate. Combine the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 1 mL dry dichloromethane, add 1 mg p-aminophenol, add 150 μL triethylamine, add at least a 3-fold molar excess of 2-bromo-1-methylpyridinium iodide, heat at 60° for 30 min, cool, concentrate under a stream of nitrogen, add 1 mL 100 mM HCl, add 1 mL ethyl acetate, shake vigorously, centrifuge at 2500 rpm for 5 min, inject an aliquot of the supernatant. (Prepare 2-bromo-1-methylpyridinium iodide by analogy with the preparation of 2-chloro-1-methylpyridinium iodide. Add 15 g methyl iodide to 13.9 g 2-bromopyridine in 3 mL acetone at 0°, stir at room temperature for 3 days. Filter the precipitate and wash it with 50 mL dry ether, dry under reduced pressure to give 2-bromo-1-methylpyridinium iodide (Bull. Chem. Soc. Japan 1977, 50, 1863).)

HPLC VARIABLES

Column: 250 × 4.6 10 μm Nucleosil C-18
Mobile phase: MeOH:water:perchloric acid 75:25:0.1 containing 50 mM sodium perchlorate
Column temperature: 25 ± 0.1
Flow rate: 0.9
Detector: E, 0.75 v, Ag/AgCl reference electrode

CHROMATOGRAM

Retention time: 15.5
Limit of detection: 2 ng

OTHER SUBSTANCES

Extracted: cholic acid, deoxycholic acid, lithocholic acid

KEY WORDS

derivatization

REFERENCE

Ikenoya,S.; Hiroshima,O.; Ohmae,M.; Kawabe,K. Electrochemical detector for high performance liquid chromatography. IV. Analysis of fatty acids, bile acids and prostaglandins by derivatization to an electrochemically active form, *Chem.Pharm.Bull.(Tokyo)*, **1980**, *28*, 2941–2947.

SAMPLE

Matrix: bile

Sample preparation: Extract bile with 20 volumes EtOH, boil on a hot water bath, cool, let stand overnight, filter (Toyo Roshi 5A paper), filter (0.45 μm), add 200 μg/mL testosterone acetate in EtOH (final IS concentration 100 μg/mL), inject a 5-10 μL aliquot.

HPLC VARIABLES

Guard column: Bondapak C18/Corasil
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:MeOH:30 mM phosphate buffer 10:60:30, pH 3.40
Flow rate: 0.5

Injection volume: 5-10
Detector: UV 200

CHROMATOGRAM

Retention time: 25 (taurine conjugate), 30 (glycine conjugate)
Internal standard: testosterone acetate (39)
Limit of detection: 50 ng

OTHER SUBSTANCES

Extracted: ursodiol, conjugates, bile acids, deoxycholic acid

REFERENCE

Nakayama,F.; Nakagaki,M. Quantitative determination of bile acids in bile with reversed-phase high-performance liquid chromatography, *J.Chromatogr.*, **1980**, *183*, 287–293.

SAMPLE

Matrix: bile, blood
Sample preparation: Serum. 100-200 μL Serum + 1 mL MeOH, mix, sonicate for 15 min. Remove a 600 μL aliquot of the supernatant and evaporate it to dryness under a stream of nitrogen, reconstitute with 1 mL 50 mM pH 7.0 phosphate buffer, add to a Sep-Pak C18 SPE cartridge, wash with 2 mL MeOH:water 20:80, elute with 4 mL MeOH:water 80:20. Evaporate the eluate to dryness under reduced pressure at 40°, reconstitute with 1 mL MeOH. Remove a 500 μL aliquot and add it to 50 μL 100 μM lauric acid in MeOH, add 50 μL 0.1 mg/mL KOH on MeOH, evaporate to dryness under a stream of nitrogen, add 100 μL 1 mg/mL dicyclohexyl-18-crown-6 in MeCN, add 100 μL 25 mM 1-bromoacetylpyrene in MeCN, mix, heat at 40° for 30 min, cool, inject an 8 μL aliquot. Bile. Mix 10 μL bile with 10 mL 50 mM pH 7.0 phosphate buffer, add a 1 mL aliquot to a Sep-Pak C18 SPE cartridge, wash with 2 mL MeOH:water 20:80, elute with 4 mL MeOH:water 80:20. Evaporate the eluate to dryness under reduced pressure at 40°, reconstitute with 1 mL MeOH. Remove a 500 μL aliquot and add it to 50 μL 100 μM lauric acid in MeOH, add 50 μL 0.1 mg/mL KOH on MeOH, evaporate to dryness under a stream of nitrogen, add 100 μL 1 mg/mL dicyclohexyl-18-crown-6 in MeCN, add 100 μL 25 mM 1-bromoacetylpyrene in MeCN, mix, heat at 40° for 30 min, cool, inject an 8 μL aliquot.

HPLC VARIABLES

Column: 100 × 8 10 μm Model RCM-100 Radial-Pak A (Waters)
Mobile phase: Gradient. MeCN:MeOH:water 100:50:40 for 30 min then 100:50:20 (step gradient).
Flow rate: 2
Injection volume: 8
Detector: F ex 370 em 440

CHROMATOGRAM

Retention time: 44
Internal standard: lauric acid (56)
Limit of detection: 10 pmole
Limit of quantitation: 50 pmole

OTHER SUBSTANCES

Extracted: cholic acid, deoxycholic acid, glycochenodeoxycholic acid, glycocholic acid, glycodeoxycholic acid, glycolithocholic acid, glycoursodeoxycholic acid, lithocholic acid, ursodiol

KEY WORDS

derivatization; serum; SPE

REFERENCE

Kamada,S.; Maeda,M.; Tsuji,A. Fluorescence high-performance liquid chromatographic determination of free and conjugated bile acids in serum and bile using 1-bromoacetylpyrene as a pre-labeling reagent, *J.Chromatogr.*, **1983**, *272*, 29–41.

SAMPLE

Matrix: bile, blood, feces, gastric contents, tissue

Sample preparation: Condition a Sep-Pak C18 cartridge with 2 mL 720 mM MeOH in water and 6 mL 100 mM pH 7.0 potassium phosphate buffer. Serum. 200 μL Serum + 1 mL MeCN, mix, sonicate for 10 min, centrifuge at 17000 g for 15 min. Remove a 600 μL aliquot of the supernatant and evaporate it to dryness under a stream of nitrogen at 75°, reconstitute with 5 mL 100 mM pH 7.0 potassium phosphate buffer. Add to the SPE cartridge at 0.5 mL/min, wash with 2 mL 40 mM MeOH in water, elute with 4 mL 720 mM MeOH in water, filter (0.45 μm), evaporate the filtrate to dryness, reconstitute with 50 μL 250 μM lauric acid in MeOH, add 50 μL 1.8 mM KOH in MeOH, evaporate to dryness under a stream of nitrogen at 75°, reconstitute with 100 μL 10 mM 4-bromomethyl-7-methoxycoumarin in MeCN containing 5 mM dicyclohexyl-18-crown-6, let stand at room temperature for 35 min, inject an aliquot. Liver. Homogenize (glass homogenizer) liver in 1 mL 720 mM EtOH in water, add 2 mL 720 mM EtOH in water, heat at 75° for 15 min, centrifuge at 17000 g for 10 min, remove the supernatant, extract the residue twice more. Combine the supernatants and evaporate them to dryness at 75°, reconstitute with 5 mL 100 mM pH 7.0 potassium phosphate buffer. Add to the SPE cartridge at 0.5 mL/min, wash with 2 mL 40 mM MeOH in water, elute with 4 mL 720 mM MeOH in water, filter (0.45 μm), evaporate the filtrate to dryness, reconstitute with 50 μL 250 μM lauric acid in MeOH, add 50 μL 1.8 mM KOH in MeOH, evaporate to dryness under a stream of nitrogen at 75°, reconstitute with 100 μL 10 mM 4-bromomethyl-7-methoxycoumarin in MeCN containing 5 mM dicyclohexyl-18-crown-6, let stand at room temperature for 35 min, inject an aliquot. Bile. Dilute 20 μL bile with 10 mL 100 mM pH 7.0 potassium phosphate buffer. Add 1 mL to the SPE cartridge at 0.5 mL/min, wash with 2 mL 40 mM MeOH in water, elute with 4 mL 720 mM MeOH in water, filter (0.45 μm), evaporate the filtrate to dryness, reconstitute with 50 μL 250 μM lauric acid in MeOH, add 50 μL 1.8 mM KOH in MeOH, evaporate to dryness under a stream of nitrogen at 75°, reconstitute with 100 μL 10 mM 4-bromomethyl-7-methoxycoumarin in MeCN containing 5 mM dicyclohexyl-18-crown-6, let stand at room temperature for 35 min, inject an aliquot. Gastric juice. Dilute 1 mL gastric juice with 9 mL 100 mM pH 7.0 potassium phosphate buffer, sonicate for 10 min. Add 1 mL to the SPE cartridge at 0.5 mL/min, wash with 2 mL 40 mM MeOH in water, elute with 4 mL 720 mM MeOH in water, filter (0.45 μm), evaporate the filtrate to dryness, reconstitute with 50 μL 250 μM lauric acid in MeOH, add 50 μL 1.8 mM KOH in MeOH, evaporate to dryness under a stream of nitrogen at 75°, reconstitute with 100 μL 10 mM 4-bromomethyl-7-methoxycoumarin in MeCN containing 5 mM dicyclohexyl-18-crown-6, let stand at room temperature for 35 min, inject an aliquot. Feces. Dilute 1 g feces with 9 mL MeOH, mix thoroughly, sonicate for 10 min, centrifuge at 17000 g for 10 min. Remove a 1 mL aliquot of the supernatant and evaporate it to dryness, reconstitute with 5 mL 100 mM pH 7.0 potassium phosphate buffer. Add to the SPE cartridge at 0.5 mL/min, wash with 2 mL 40 mM MeOH in water, elute with 4 mL 720 mM MeOH in water, filter (0.45 μm), evaporate the filtrate to dryness, reconstitute with 50 μL 250 μM lauric acid in MeOH, add 50 μL 1.8 mM KOH in MeOH, evaporate to dryness under a stream of nitrogen at 75°, reconstitute with 100 μL 10 mM 4-bromomethyl-7-methoxycoumarin in MeCN containing 5 mM dicyclohexyl-18-crown-6, let stand at room temperature for 35 min, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Ultrasphere I.P. C18

Mobile phase: Gradient. A was MeCN:MeOH:water 100:50:75. B was MeCN:MeOH 100:50. A:B 100:0 for 7 min, to 70:30 over 0.5 min, maintain at 70:30 for 5 min, to 50:50 over 0.5 min, maintain at 50:50 over 7 min, to 25:75 over 1 min, maintain at 25:75 for 7 min.

Column temperature: 35

Flow rate: 1.7

Injection volume: 100
Detector: F

CHROMATOGRAM
Retention time: 21.3
Internal standard: lauric acid (24.5)
Limit of detection: 0.5 pmole

OTHER SUBSTANCES
Extracted: cholic acid, deoxycholic acid, glycinechenodeoxycholic acid, glycinecholic acid, glycinedeoxycholic acid, glycinelithocholic acid, glycineursodeoxycholic acid, lithocholic acid, ursodiol (ursodeoxycholic acid)

KEY WORDS
derivatization; SPE; liver; serum

REFERENCE
Güldütuna,S.; You,T.; Kurts,W.; Leuschner,U. High performance liquid chromatographic determination of free and conjugated bile acids in serum, liver biopsies, bile, gastric juice and feces by fluorescence labeling, *Clin.Chim.Acta*, **1993**, *214*, 195–207.

SAMPLE
Matrix: bile, blood, urine
Sample preparation: Urine. Condition a Bond Elut C18 SPE cartridge with MeOH and water. Dilute 100-200 μL urine 1:4 with 100 mM NaOH, add to the SPE cartridge, wash with water, elute with MeOH, evaporate the eluate, reconstitute the residue in mobile phase, inject an aliquot. Serum. Condition a Bond Elut C18 SPE cartridge with MeOH and water. Dilute 100-500 μL serum with 3.5 mL 100 mM NaOH, heat at 64° for 30 min, add to the SPE cartridge, wash with water, elute with MeOH, evaporate the eluate, reconstitute the residue in mobile phase, inject an aliquot. Bile. Dilute 1:500 to 1:1000 with mobile phase, filter (0.22 μm, inject an aliquot.

HPLC VARIABLES
Column: 70 × 4.6 3 μm Ultrasphere XL C18
Mobile phase: MeOH:15 mM ammonium acetate 80:20, apparent pH 6.0 ± 0.1
Flow rate: 0.3
Detector: MS, electrospray, Fisons VG TRIO 2000 quadrupole (6% of the mobile phase was diverted to the MS detector) or evaporative light scattering detector (Varex)

CHROMATOGRAM
Retention time: 17.60
Limit of detection: 15 pg

OTHER SUBSTANCES
Extracted: deoxycholic acid, ursodiol, bile acids, cholic acid, glycochenodeoxycholic acid, glycocholic acid, glycodeoxycholic acid, glycoursodeoxycholic acid, lithocholic acid, taurochenodeoxycholic acid, taurocholic acid, taurodeoxycholic acid, tauroursodeoxycholic acid

KEY WORDS
serum; SPE; hamster; human; LC-MS

REFERENCE
Roda,A.; Gioacchini,A.M.; Cerrè,C.; Baraldini,M. High-performance liquid chromatographic-electrospray mass spectrometric analysis of bile acids in biological fluids, *J.Chromatogr.B*, **1995**, *665*, 281–294.

SAMPLE
Matrix: bile, formulations

Sample preparation: Bile. Condition a 200 mg Bond Elut C18 SPE cartridge with 5 mL MeOH and 5 mL water. Condition a 500 mg Bond Elut SAX SPE cartridge with 5 mL MeOH, 5 mL water, and 5 mL MeOH. 50 μL Bile + 5 mL 50 mM pH 7.5 phosphate buffer, vortex, add to the C18 SPE cartridge, wash with 5 mL MeOH:40 mM pH 4.3 acetate buffer 40:60, wash with 10 mL water, elute with 2 mL MeOH. Add the eluate to the SAX SPE cartridge, elute with 3.5 mL MeOH, collect all the effluent from the cartridge (J. Pharm. Biomed. Anal. 1990, 8, 235). Evaporate to dryness under a stream of nitrogen, reconstitute with 2 mL MeOH, sonicate at 40° for 3 min, filter (0.2 μm). Add a 500 μL aliquot of the filtrate to 50 μL 0.01% KOH in MeOH, evaporate to dryness, reconstitute with 200 μL MeOH:water 10:90, sonicate at 40° for 3 min, add 300 μL 20 mM tetrahexylammonium bromide in 100 mM pH 7.0 phosphate buffer, add 50 μL 2.1 mg/mL 2-bromoacetyl-6-methoxynaphthalene in acetone, sonicate at 40° for 10 min, add 50 μL 43.6 μg/mL IS in MeOH:water 75:25, add 300 μL MeCN, sonicate at room temperature for 1 min, inject a 50 μL aliquot. Formulations. Powder capsule contents, weigh out amount containing about 25 mg compound, add 100 mL MeOH (water for bile acid salts), stir for 10 min, filter, dilute the filtrate 10-fold with water (or MeOH:water 10:90 for bile acid salts). Evaporate 50 μL 0.01% KOH in MeOH in to a tube, add a 200 μL aliquot of the diluted filtrate, add 300 μL 20 mM tetrahexylammonium bromide in 100 mM pH 7.0 phosphate buffer, add 50 μL 2.1 mg/mL 2-bromoacetyl-6-methoxynaphthalene in acetone, sonicate at 40° for 10 min, add 50 μL 43.6 μg/mL IS in MeOH:water 75:25, add 300 μL MeCN, sonicate at room temperature for 1 min, inject a 50 μL aliquot. (Prepare 2-bromoacetyl-6-methoxynaphthalene by stirring equimolar amounts of 2-acetyl-6-methoxynaphthalene (Janssen Chimica, Belgium) and phenyltrimethylammonium tribromide in THF at room temperature for 3 h (Phosphorus and Sulfur 1985, 25, 357), purify by column chromatography on silica gel with chloroform:petroleum ether 50:50 (mp 109-112°) (Chromatographia 1992, 33, 13).)

HPLC VARIABLES

Column: 250 × 4.6 5 μm Hypersil RP-18

Mobile phase: Gradient. For bile use MeCN:water 60:40 for 10 min, to 80:20 over 10 min, maintain at 80:20 for 25 min, return to initial conditions over 5 min. For formulations use isocratic MeCN:water 78:22.

Flow rate: 1

Injection volume: 50

Detector: F ex 300 em 460

CHROMATOGRAM

Retention time: 26.5 (gradient), 12 (isocratic)

Internal standard: 6-methoxynaphthacyl ester of valproic acid (23 (gradient), 10.5 (isocratic))

Limit of detection: 1-2 pmole

OTHER SUBSTANCES

Extracted: cholic acid, deoxycholic acid, glycochenodeoxycholic acid, glycocholic acid, glycolithocholic acid, glycoursodeoxycholic acid, lithocholic acid, ursodiol

KEY WORDS

derivatization; capsules; SPE

REFERENCE

Cavrini,V.; Gatti,R.; Roda,A.; Cerrè,C.; Roveri,P. HPLC-fluorescence determination of bile acids in pharmaceuticals and bile after derivatization with 2-bromoacetyl-6-methoxynaphthalene, *J.Pharm.Biomed.Anal.*, **1993**, *11*, 761–770.

SAMPLE

Matrix: bile, gastric contents

Sample preparation: Condition a 200 mg Bond Elut C18 SPE cartridge with 5 mL MeOH and 5 mL water. Condition a 500 mg Bond Elut SAX SPE cartridge with 5 mL MeOH, 5

mL water, and 5 mL MeOH. Mix 50 μL bile or 500 μL gastric juice with 5 mL 50 mM pH 7.5 phosphate buffer, vortex, add to the C18 SPE cartridge, wash with 5 mL MeOH: 40 mM pH 4.3 acetate buffer 40:60, wash with 10 mL water, elute with 2 mL MeOH. Add the eluate to the SAX SPE cartridge, elute with 3.5 mL MeOH, collect all the effluent from the cartridge. Evaporate to dryness under a stream of nitrogen, reconstitute with 200 μL initial mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Ultrasphere ODS
Mobile phase: Gradient. A was MeOH:30 mM sodium acetate 65:35, adjusted to pH 4.3 with phosphoric acid. B was MeOH:70 mM sodium acetate 90:10, adjusted to pH 4.3 with phosphoric acid. A:B 85:15 for 10 min, to 10:90 over 25 min, maintain at 10:90 for 5 min.
Flow rate: 1
Injection volume: 20
Detector: UV 210

CHROMATOGRAM
Retention time: 33

OTHER SUBSTANCES
Extracted: cholic acid, deoxycholic acid, lithocholic acid, ursodiol

KEY WORDS
SPE

REFERENCE
Scalia,S. Group separation of free and conjugated bile acids by pre-packed anion-exchange cartridges, *J.Pharm.Biomed.Anal.*, **1990**, *8*, 235–241.

SAMPLE
Matrix: blood
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 2 mL MeOH, 10 mL water, and 2 mL 100 mM pH 8.0 Tris-HCl buffer. 5-7 mL Serum + 19 volumes 100 mM pH 8.0 Tris-HCl buffer, sonicate for 10 min, add to the SPE cartridge, wash with 15 mL water, elute with 6-7 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 50°, dissolve residue in water, filter (Millipore GS 0.22 μm), wash filter, evaporate filtrates to dryness, reconstitute in 100 μL mobile phase, inject a 30 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeOH:20 mM KH_2PO_4 65:35, adjust pH to 5.3
Flow rate: 1.4
Injection volume: 30
Detector: UV 210

CHROMATOGRAM
Retention time: 15 (taurine conjugate), 21 (glycine conjugate)
Limit of detection: 40 nM (glycine conjugate), 30 nM (taurine conjugate)

OTHER SUBSTANCES
Extracted: conjugates, ursodiol, deoxycholic acid, bile acids

KEY WORDS
serum; SPE

REFERENCE

Linnet,K. A high-pressure liquid chromatogaphic-enzymatic assay for glycine and taurine conjugates of cholic, chenodeoxycholic and deoxycholic acid in serum, *Scand.J.Clin.Lab.Invest.*, **1982**, *42*, 455–460.

SAMPLE

Matrix: blood

Sample preparation: Condition a BondElut SPE cartridge with 5 mL EtOH and 5 mL water. 100 μL Serum + 250 ng deoxycholic acid 12-propionate + 1 mL 500 mM pH 7.0 phosphate buffer, mix, add to the SPE cartridge, wash with 2 mL water, wash with 1 mL 1.5% EtOH, elute with 2 mL 90% EtOH. Evaporate a 400 μL aliquot of the eluate, add 100 μL 2 mg/mL 1-anthroyl nitrile in MeCN, add 0.16% quinuclidine in MeCN, heat at 60° for 20 min, add 50 μL MeOH, evaporate under nitrogen. Dissolve the residue in 1 mL 90% EtOH, add to a 18 × 6 100 mg column of PHP-LH-20 Sephadex at 0.2 mL/min, wash with 1 mL 90% EtOH, elute with 5 mL 100 mM acetic acid in 90% EtOH (free bile acids), elute with 5 mL 200 mM formic acid in 90% EtOH (glycine-conjugated bile acids), elute with 5 mL 300 mM pH 6.3 acetic acid-potassium acetate in 90% EtOH (taurine-conjugated bile acids). Evaporate each fraction, dissolve the residue in 100-200 μL MeOH, inject a 5-10 μL aliquot. (Preparation of PHP-LH-20 Sephadex is as follows. Suspend 75.7 g Sephadex LH-20 in 200 mL dichloromethane using a glass stirring rod (not a magnetic stirrer) for 30 min, add 19 mL boron trifluoride ethyl etherate, after 15 min add 50 mL 35% epichlorohydrin in dichloromethane at 1-2 mL/min (Caution! Epichlorohydrin is a carcinogen!), stir for another 30 min, filter, wash with EtOH, dry chlorohydroxypropyl Sephadex LH-20 at 50° (J.Chromatogr. 1971, 59, 45). Stir 27.2 g chlorohydroxypropyl Sephadex LH-20 in 100.5 mL piperidine at room temperature for 30 min, add 5.74 g KOH in 302 mL MeOH, heat at 50-60° for 3 h with occasional shaking, filter, wash with EtOH:water 50:50, wash with 200 mM acetic acid in EtOH:water 70:30, wash with EtOH:water 90:10 until washings become neutral, store in EtOH:water 90:10 (Clin. Chim. Acta 1978 87 141). Prepare 1-anthroyl nitrile as follows. Dissolve 50 g benzanthrone in 500 mL concentrated sulfuric acid with gentle warming, pour this solution cautiously into 4 L hot water with vigorous stirring. Boil the suspension and slowly add 200 g chromium(VI) oxide (Caution! Chromium oxide is a carcinogen and highly corrosive!), after 6 h cool the mixture, filter, wash the precipitate with hot water. Dissolve the precipitate in dilute ammonia and precipitate with acid, crystallize from boiling concentrated nitric acid to give anthraquinone-1-carboxylic acid (Ber. 1924, 57, 1775). Warm, on a water bath, anthraquinone-1-carboxylic acid in dilute ammonia with twice the amount of zinc dust, when the reaction has ceased (30 min ?) filter the reaction the reaction mixture, add HCl to the filtrate to obtain anthracene-1-carboxylic acid as yellow needles, recrystallize from EtOH (mp 245°) (Ber 1897, 30, 1118). Stir 1 g anthracene-1-carboxylic acid in 15 mL anhydrous dichloromethane, add 2 mL oxalyl chloride, reflux for 1 h, evaporate to give 1-anthroyl chloride as an oily residue. Dissolve 1-anthroyl chloride in 15 mL dichloromethane, add 3 mL trimethylsilyl cyanide, add 1 mg zinc iodide, stir at room temperature for 2 h, evaporate to dryness, recrystallize from hexane/dichloromethane to give 1-anthroyl nitrile as orange-yellow needles (mp 164-5°) (Anal.Chim.Acta 1983, 147, 397).)

HPLC VARIABLES

Column: 150 × 4 5 μm Cosmosil 5C18
Mobile phase: MeOH:0.3% pH 6.0 potassium phosphate buffer 5:1
Flow rate: 1.8
Injection volume: 10
Detector: F ex 370 em 470

CHROMATOGRAM

Retention time: 13
Internal standard: deoxycholic acid 12-propionate (20)
Limit of detection: 50 nM

OTHER SUBSTANCES

Extracted: ursodiol, cholic acid deoxycholic acid, conjugates

KEY WORDS
serum; SPE; derivatization

REFERENCE
Goto,J.; Saito,M.; Chikai,T.; Goto,N.; Nambara,T. Studies on Steroids. CLXXXVII. Determination of serum bile acids by high-performance liquid chromatography with fluorescence labeling, *J.Chromatogr.*, **1983**, *276*, 289–300.

SAMPLE
Matrix: blood
Sample preparation: Add 1 mL serum to a Waters C18 SPE cartridge, wash with two 4 mL portions of water, wash with two 2 mL portions of MeOH:water 10:90, wash with two 2 mL portions of MeOH:water 20:80, wash with two 2 mL portions of MeOH:water 30:70, wash with two 2 mL portions of MeOH:water 50:50, elute with 3 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 80°, reconstitute the residue in 50 μL water, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 5 μm Lichrosorb RP 18
Mobile phase: MeOH:30 mM KH_2PO_4 76:24
Flow rate: 1.2
Injection volume: 20
Detector: UV 201

CHROMATOGRAM
Retention time: 16
Limit of detection: 50 ng/mL

OTHER SUBSTANCES
Extracted: ursodiol (ursodeoxycholic acid)

KEY WORDS
serum; SPE

REFERENCE
Baillet-Guffroy,A.; Baylocq,D.; Rabaron,A.; Pellerin,F. Nuclear magnetic resonance spectrometry and liquid chromatography of two bile acid epimers: ursodeoxycholic and chenodeoxycholic acid, *J.Pharm.Sci.*, **1984**, *73*, 847–849.

SAMPLE
Matrix: blood
Sample preparation: Deproteinize 20 μL serum with a pretreatment column (Autoserumout, Sekisui), inject an aliquot.

HPLC VARIABLES
Column: 150 × 4.6 Medipola Bile column (Sekisui)
Mobile phase: Gradient. A was MeCN:MeOH:30 mM ammonium acetate 20:20:60. B was MeCN:MeOH:30 mM ammonium acetate 30:30:40. A:B from 100:0 to 80:20 over 10 min, to 0:100 over 27 min, maintain at 0:100 for 30 min.
Flow rate: 1
Detector: F ex 340 em 460 following post-column reaction detection. The effluent from the column was mixed with reagent pumped at 1 mL/min, the mixture flowed through a 20 × 4 3α-HSD column (Sekisui) containing bound 3α-hydroxysteroid dehydrogenase to the detector. (The reagent was 1.36 g/L KH_2PO_4, 372 mg/L disodium EDTA, 140 mg/L βNAD, and 450 μL/L 2-mercaptoethanol in water adjusted to pH 7.8 with 5 M KOH.)

CHROMATOGRAM
Retention time: 34

OTHER SUBSTANCES
Extracted: ursodiol, deoxycholic acid, bile acids

KEY WORDS
post-column reaction; immobilized enzyme reactor; serum

REFERENCE
Adachi,Y.; Nanno,T.; Itoh,T.; Kurumi,Y.; Yamazaki,K.; Sawada,Y.; Yamamoto,T. Determination of individual serum bile acids in chronic liver diseases: fasting levels and results of oral chenodeoxycholic acid tolerance test, *Gastroenterol.Jpn.*, **1988**, *23*, 401–407.

SAMPLE
Matrix: blood
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 5 mL MeOH and 5 mL water. Dilute 100-200 μL serum with 4 mL 400 mM sodium bicarbonate, add to the SPE cartridge, wash with 20 mL water, elute with 2 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 45°, reconstitute the residue in 100 μL 2 mg/mL 4-bromomethyl-7-methoxycoumarin in MeCN, add 400 μg sodium carbonate, add 50 μL 20 mg/mL 18-crown-6 in MeCN, heat at 40° for 1 h, make up to 500 μL with MeCN, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 5 μm Nova-Pak ODS
Mobile phase: Gradient. A was MeCN:MeOH:water 15:13.8:71.2. B was MeCN. A:B from 100:0 to 37:63 over 47 min (Waters convex curve + 2), to 0:100 over 0.1 min (Waters curve +9), maintain at 0:100 for 7.9 min, re-equilibrate at initial conditions for 6 min.
Flow rate: 1 for 47 min then 1.5
Injection volume: 10
Detector: F ex 320 em 385

CHROMATOGRAM
Retention time: 45.23
Limit of detection: 80 nM

OTHER SUBSTANCES
Extracted: cholic acid, deoxycholic acid, glycochenodeoxycholic acid, glycocholic acid, glycodeoxycholic acid, glycolithocholic acid, ursodiol

KEY WORDS
derivatization; serum; SPE

REFERENCE
Wang,G.F.; Stacey,N.H.; Earl,J. Determination of individual bile acids in serum by high performance liquid chromatography, *Biomed.Chromatogr.*, **1990**, *4*, 136–140.

SAMPLE
Matrix: bulk
Sample preparation: Dissolve 1-5 mg bile acid in 500 μL chloroform with enough MeOH to make a solution, add a solution of 1-naphthyldiazomethane in ether until the reddish-orange color persists, if the color disappears within 1 h add more reagent, add 1 drop acetic acid to decompose excess reagent, make up to 1 mL, inject a 5 μL aliquot. (Preparation of 1-naphthyldiazomethane is as follows. Stir 6.7 g 1-naphthaldehyde and 8.5 g 80% hydrazine hydrate in 150 mL EtOH at room temperature for 3 h (Caution! Hydrazine hydrate is a carcinogen!). Remove the solid by filtration and recrystallize it twice from EtOH to give 1-naphthaldehyde hydrazone as white crystals (mp 91-92°). Stir 3.1 g 1-naphthaldehyde hydrazone, 5 g anhydrous sodium sulfate, 50 mL ether, 1 mL EtOH saturated with KOH, and 10 g yellow mercuric oxide for 5 h, filter (sintered glass), con-

centrate the filtrate under reduced pressure to give 1-naphthyldiazomethane as red crystals (mp 40-41°) (Bull. Chem. Soc. Japan 1967, 40, 691).)

HPLC VARIABLES
Column: 300 mm long μPorasil
Mobile phase: Hexane:THF:MeOH 75:30:2
Flow rate: 1
Injection volume: 5
Detector: UV 280

CHROMATOGRAM
Retention time: 12
Limit of detection: 20-30 ng

OTHER SUBSTANCES
Simultaneous: deoxycholic acid, 3,7-dihydroxy-12-ketocholanic acid, lithocholic acid

KEY WORDS
derivatization; normal phase

REFERENCE
Matthees,D.P.; Purdy,W.C. Naphthyldiazomethane as a derivatizing agent for the high-performance liquid chromatography detection of bile acids, *Anal.Chim.Acta*, **1979**, *109*, 161–164.

SAMPLE
Matrix: bulk
Sample preparation: Add 5 mL of a 39.2 mg/mL solution in dry MeCN to 120 mg 1-(2,5-dihydroxyphenyl)-2-bromoethane and 100 μL triethylamine, heat at 70° for 2 h, dilute with 20 mL water, extract 3 times with diethyl ether. Combine the extracts and wash them with saturated sodium bicarbonate and water, dry over anhydrous sodium sulfate, evaporate, reconstitute, inject a 5 μL aliquot. (Preparation of 1-(2,5-dihydroxyphenyl)-2-bromoethane is as follows. Slowly add 2.5 g phenyltrimethylammonium tribromide to a solution of 2',5'-dihydroxyacetophenone in 20 mL dry THF, stir at room temperature overnight (check by TLC with cyclohexane:ethyl acetate 70:30). Remove the precipitate by filtration and dry under reduced pressure, chromatograph using cyclohexane:ethyl acetate 70:30 to give 1-(2,5-dihydroxyphenyl)-2-bromoethane.)

HPLC VARIABLES
Guard column: 4 × 4 5 μm 5 μm Hypersyl ODS RP-18
Column: 100 × 4.6 3 μm Adsorbosphere
Mobile phase: MeCN:MeOH:100 mM pH 6.5 sodium acetate buffer 20:60:20
Flow rate: 1
Injection volume: 5
Detector: E, ESA Coulochem Model 5100A, Model 5010 analytical cell, porous graphite electrodes +0.6 V

CHROMATOGRAM
Retention time: 8.71
Limit of detection: 0.78 nM

OTHER SUBSTANCES
Simultaneous: ursodiol

KEY WORDS
derivatization

REFERENCE
Bousquet,E.; Santagati,N.A.; Tirendi,S. Determination of chenodeoxycholic acid in pharmaceutical preparations of ursodeoxycholic acid by high performance liquid chromatography with coulometric electrochemical detection, *J.Liq.Chromatogr.Rel.Technol.*, **1997**, *20*, 757–770.

SAMPLE
Matrix: formulations
Sample preparation: Powder capsule contents, weigh out amount containing 25.5 mg chenodiol, add 100 mL acetone:MeCN 40:60, stir for 10 min, filter (paper), dilute with MeCN to a final concentration of 25.5 μg/mL. Mix 500 μL of this solution with 300 μL 1.28 mg/mL 2-bromoacetyl-6-methoxynaphthalene in MeCN, add 50 μL 3% triethylamine in MeCN, heat at 70° for 30 min, cool, add 50 μL 28 μg/mL IS in MeCN, inject a 50 μL aliquot. (Prepare 2-bromoacetyl-6-methoxynaphthalene by stirring equimolar amounts of 2-acetyl-6-methoxynaphthalene (Janssen Chimica, Belgium) and phenyltrimethylammonium tribromide in THF at room temperature for 3 h (Phosphorus and Sulfur 1985, 25, 357), purify by column chromatography on silica gel with chloroform:petroleum ether 50:50 (mp 109-112°).)

HPLC VARIABLES
Column: 150 × 4.6 5 μm Hypersil RP-18
Mobile phase: Gradient. MeCN:water from 55:45 to 80:20 over 20 min, maintain at 80:20 for 10 min, return to initial conditions over 10 min. Alternatively, isocratic at MeCN:water 70:30.
Flow rate: 1
Injection volume: 50
Detector: F ex 300 em 460

CHROMATOGRAM
Retention time: 18 (gradient), 7.5 (isocratic)
Internal standard: 6-methoxynaphthacyl ester of valproic acid (Dissolve 2 mmole valproic acid and 1 mmole 2-bromoacetyl-6-methoxynaphthalene in 10 mL anhydrous MeCN, add 500 μL triethylamine, heat to 60° for 30 min, cool, add 30 mL water, extract three times with 10 mL portions of diethyl ether. Combine the organic layers and wash them with 5% sodium bicarbonate solution, wash three times with 10 mL portions of water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize from water to give 6-methoxynaphthacyl ester of valproic acid (mp 56-7°).) (5 (isocratic))
Limit of detection: 2-3 pmole

OTHER SUBSTANCES
Simultaneous: cholic acid, deoxycholic acid, glycochenodeoxycholic acid, glycocholic acid, lithocholic acid

KEY WORDS
derivatization; capsules

REFERENCE
Gatti,R.; Cavrini,V.; Roveri,P. 2-Bromoacetyl-6-methoxynaphthalene: A useful fluorescent labelling reagent for HPLC analysis of carboxylic acids, *Chromatographia*, **1992**, *33*, 13–18.

SAMPLE
Matrix: solutions
Sample preparation: Mix an aliquot of solution (or hydrolyzed bile) with a 50% molar excess of triethylamine in MeCN, warm briefly, add a 50% molar excess of 100 mM 2-bromoacetophenone in MeCN, heat at 80-90° for 45-60 min, evaporate to dryness, reconstitute with dioxane (Caution! Dioxane is a carcinogen!), filter (0.47 μm), inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Partisil 10/25 ODS

Mobile phase: Gradient. n-Heptane:dioxane 90:10 for 3 min then n-heptane:dioxane:isopropanol 70:25:5 (step gradient). (Caution! Dioxane is a carcinogen!)
Flow rate: 1.2
Detector: UV 254

CHROMATOGRAM
Retention time: 18
Limit of quantitation: 5 pmole

OTHER SUBSTANCES
Simultaneous: cholic acid, deoxycholic acid, hyodeoxycholic acid, lithocholic acid, ursodiol

KEY WORDS
derivatization

REFERENCE
Stellaard,F.; Hachey,D.L.; Klein,P.D. Separation of bile acids as their phenacyl esters by high-pressure liquid chromatography, *Anal.Biochem.*, **1978**, *87*, 359–366.

SAMPLE
Matrix: solutions
Sample preparation: Treat a solution in MeOH with a slight excess of tetramethylammonium hydroxide in MeOH, evaporate to dryness under a stream of nitrogen, reconstitute with MeCN, add a 2-10 fold excess of 9-(chloromethyl)anthracene in cyclohexane, heat at 75° for 15 min, very dilute solutions may require longer times), dilute with MeCN, inject an aliquot.

HPLC VARIABLES
Column: 300 mm long "Fatty Acid" reversed-phase (Waters)
Mobile phase: MeOH:water 88:12 (A) or 82:18 (B)
Flow rate: 0.75
Detector: UV 254

CHROMATOGRAM
Retention time: 32

OTHER SUBSTANCES
Simultaneous: cholic acid, glycochenodeoxycholic acid, glycocholic acid, glycodeoxycholic acid
Interfering: deoxycholic acid

KEY WORDS
derivatization

REFERENCE
Korte,W.D. 9-(Chloromethyl)anthracene: a useful derivatizing reagent for enhanced ultraviolet and fluorescence detection of carboxylic acids with liquid chromatography, *J.Chromatogr.*, **1982**, *243*, 153–157.

SAMPLE
Matrix: solutions
Sample preparation: Mix a 100 μL aliquot of a 1-100 μM solution in 20 mM pH 7.1 phosphate buffer with 10 μL 10 mM tetrabutylammonium hydrogen sulfate in water and 100 μL 1 mM N-(9-acridinyl)bromoacetamide in chloroform, stir at 90° for 20-30 min, cool. Remove a 10 μL aliquot of the organic layer and evaporate it to dryness under reduced pressure, reconstitute with mobile phase, sonicate, inject an aliquot. (Synthesis of N-(9-acridinyl)bromoacetamide is as follows. Dissolve 2.49 g 9-aminoacridine hydrochloride

hydrate in water, add dilute NaOH to precipitate the free base, extract with ethyl acetate, dry over anhydrous magnesium sulfate, filter, evaporate to give 9-aminoacridine as yellow needle-shaped crystals (mp 239-240°). Add 1.01 g bromoacetyl bromide in 20 mL diethyl ether dropwise with stirring to 970 mg 9-aminoacridine dissolved in 50 mL acetone containing 1.02 g triethylamine, filter, wash the solid with acetone. Evaporate the filtrate and chromatograph the residue on a 260 × 30 glass column of 70-230 mesh silica gel 60 (Merck) with chloroform:ethyl acetate 2:1. Collect the strong yellow band and evaporate it to dryness, recrystallize from MeOH to give N-(9-acridinyl)bromoacetamide as light yellow crystals (mp 180-182° d).)

HPLC VARIABLES
Column: 150 × 4.6 5 μm Nucleosil C18
Mobile phase: MeCN:water:phosphoric acid 40:60:0.2
Flow rate: 1
Injection volume: 20
Detector: F ex 357.5 em 482

CHROMATOGRAM
Retention time: 36
Limit of detection: 10 fmole

OTHER SUBSTANCES
Simultaneous: cholic acid, deoxycholic acid

KEY WORDS
derivatization

REFERENCE
Allenmark,S.; Chelminska-Bertilsson,M.; Thompson,R.A. N-(9-Acridinyl)-bromoacetamide -A powerful reagent for phase-transfer-catalyzed fluorescence labeling of carboxylic acids for liquid chromatography, *Anal.Biochem.*, **1990**, *185*, 279–285.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in mobile phase to a concentration of 1 mg/mL.

HPLC VARIABLES
Column: 100 mm long 5 μm C18
Mobile phase: MeOH:10 mM KH_2PO_4 65:35, pH 7.0
Flow rate: 1
Injection volume: 0.5
Detector: UV 200

CHROMATOGRAM
Retention time: k' = 8.03

OTHER SUBSTANCES
Simultaneous: ursodiol

REFERENCE
Roda,A.; Minutello,A.; Angellotti,M.A.; Fini,A. Bile acid structure-activity relationship: evaluation of bile acid lipophilicity using 1-octanol/water partition coefficient and reverse phase HPLC, *J.Lipid Res.*, **1990**, *31*, 1433–1443.

SAMPLE
Matrix: solutions

Sample preparation: Prepare a solution in MeCN:0.8 M NaOH 8:92, inject a 25 μL aliquot.

HPLC VARIABLES

Guard column: CarboPac PA-100 (Dionex)
Column: 250 × 4 8.5 μm CarboPac PA-100 (Dionex)
Mobile phase: MeCN:water 15:85 containing 900 mM sodium acetate and 100 mM NaOH
Flow rate: 0.8
Injection volume: 25
Detector: E, Dionex PAD-2 pulsed amperometric detector, gold working electrode, V1 + 0.05 V, t1 480 ms, V2 + 0.60 V, t2 120 ms, V3 -0.60 V, t3 60 ms

CHROMATOGRAM

Retention time: 6.70

OTHER SUBSTANCES

Simultaneous: ursodiol, deoxycholic acid, cholic acid, glycocholic acid, taurocholic acid, glycodeoxycholic acid, glycodeoxychenodeoxycholic acid, ursodeoxycholic acid, taurodeoxycholic acid, taurochenodeoxycholic acid, glycolithocholic acid, lithocholic acid, taurolithocholic acid

REFERENCE

Chaplin,M.F. Analysis of bile acids and their conjugates using high-pH anion-exchange chromatography with pulsed amperometric detection, *J.Chromatogr.B*, **1995**, *664*, 431–434.

SAMPLE

Matrix: solutions
Sample preparation: Mix 200 μL of a solution of bile acids with 50 μL 2.1 mg/mL 2-bromoacetyl-6-methoxynaphthalene in acetone, add 300 μL 10 mM tetrakis(decyl)ammonium bromide in 100 mM pH 7.0 phosphate buffer, heat at 40° for with sonication 10 min, add 300 μL 5.1 μM IS in MeCN, sonicate at room temperature for 1 min, inject a 50 μL aliquot. (Prepare 2-bromoacetyl-6-methoxynaphthalene by stirring equimolar amounts of 2-acetyl-6-methoxynaphthalene (Janssen Chimica, Belgium) and phenyltrimethylammonium tribromide in THF at room temperature for 3 h (Phosphorus and Sulfur 1985, 25, 357), purify by column chromatography on silica gel with chloroform:petroleum ether 50:50 (mp 109-112°) (Chromatographia 1992, 33, 13).)

HPLC VARIABLES

Column: 250 × 4.6 Ultracarb 5 ODS
Mobile phase: Gradient. A was water. B was MeCN:MeOH 60:40. A:B 55:45 for 20 min, to 30:70 over 10 min, maintain at 30:70 for 25 min, return to initial conditions over 5 min.
Column temperature: 35
Flow rate: 1.2
Injection volume: 50
Detector: F ex 300 em 460

CHROMATOGRAM

Retention time: 29
Internal standard: 6-methoxynaphthacyl ester of lauric acid (36)
Limit of detection: 1-2 pmole

OTHER SUBSTANCES

Simultaneous: cholic acid, deoxycholic acid, lithocholic acid, ursodiol

KEY WORDS

derivatization

REFERENCE

Gatti,R.; Roda,A.; Cerre,C.; Bonazzi,D.; Cavrini,V. HPLC-fluorescence determination of individual free and conjugated bile acids in human serum, *Biomed.Chromatogr.*, **1997**, *11*, 11–15.

SAMPLE

Matrix: solutions

Sample preparation: Mix 100 μL of a solution in MeOH with 100 μL 2.5 μg/mL IS in MeOH, evaporate to dryness, add 150 μL 0.2% 3-[4-(bromomethyl)phenyl]-7-(diethylamino)-2H-1-benzopyran-2-one (3-(4-bromomethylphenyl)-7-diethylaminocoumarin) in MeCN, add crown ether solution, heat at 60° for 20 min, evaporate to dryness at room temperature, reconstitute with 500 μL chloroform, add to a 55 × 6 column of silica gel, wash with 6 mL chloroform:MeOH 200:1, elute with 5 mL chloroform:MeOH 8:1, evaporate to dryness, reconstitute with 200 μL MeOH, inject a 5 μL aliquot. (Prepare crown ether solution by adding a large excess of solid potassium bicarbonate to a 0.4% 18-crown-6 solution in MeCN, sonicate at room temperature for 10 min, centrifuge at 1000 g for 10 min, use the supernatant. Synthesize 3-[4-(bromomethyl)phenyl]-7-(diethylamino)-2H-1-benzopyran-2-one as follows. Add 18.8 g aluminum trichloride to a solution of 94 mmoles m-diethylaminophenol and 84 g triethyl orthoformate in 185 mL chloroform at room temperature, mix for 10 min, add 50 mL 10% HCl, stir, neutralize with 10% NaOH, filter through a short column of Celite, wash through with chloroform. Wash the organic layer with saturate NaCl and dry it over anhydrous magnesium sulfate, evaporate to dryness under reduced pressure, recrystallize the product from chloroform to give 4-(diethylamino)-2-hydroxybenzaldehyde (Bull.Chem.Soc.Jpn. 1985, 58, 2192). Reflux 90 g α-bromo-p-toluic acid and 25 mL concentrated sulfuric acid in 1 L MeOH for 75 min, concentrate to 200 mL under reduced pressure, dilute with 3 mL water, recrystallize from MeOH to give methyl α-bromo-p-toluate as colorless needles (mp 54-55°). Add 35 g NaCN in 85 mL water over 10 min to a stirred solution of 70 g methyl α-bromo-p-toluate in 350 mL MeOH, heat at 50° for 30 min, dilute to 1 L with cold water, filter, recrystallize from light petroleum:benzene 2:1 (Caution! Benzene is a carcinogen!) with the aid of charcoal to give methyl α-cyano-p-toluate as colorless needles (mp 63-64°). Reflux 500 mg methyl α-cyano-p-toluate in 12 mL 10% NaOH in water for 30 min, filter, acidify the filtrate. Collect the precipitate and recrystallize it from glacial acetic acid to give 4-carboxyphenylacetic acid (homoterephthalic acid) (mp 239-241°) (J. Org. Chem. 1952, 17, 1035). Reflux 4-carboxyphenylacetic acid in MeOH in the presence of sulfuric acid to obtain methyl 4-carbomethoxyphenyl acetate (bp 172-175°/20 mm Hg) (J. Indian Chem. Soc. 1987, 64, 34). Heat 8.69 g 4-(diethylamino)-2-hydroxybenzaldehyde, 15.6 g methyl 4-carbomethoxyphenyl acetate, 2 mL piperidine, and 68 mL pyridine at 100° for 15 h, evaporate to dryness, recrystallize from ethyl acetate/hexane to give methyl 4-[7-(diethylamino)-2-oxo-2H-1-benzopyran-3-yl]benzoate as reddish-yellow prisms (mp 179-180.5). (A second crop can be obtained from the mother liquor by chromatography on silica gel using chloroform:hexane:acetone 20:18:1.) Suspend 7.1 g methyl 4-[7-(diethylamino)-2-oxo-2H-1-benzopyran-3-yl]benzoate in 240 mL 6 M HCl, reflux for 19 h, evaporate most of the solvent under reduced pressure, neutralize the residue with a saturated aqueous solution of sodium bicarbonate, filter, recrystallize from acetone to give 4-[7-(diethylamino)-2-oxo-2H-1-benzopyran-3-yl]benzoic acid as reddish-yellow prisms (mp 282-283.5°). Add 2.2 mL ethyl chloroformate and 3.2 mL triethylamine to a solution of 1.69 g 4-[7-(diethylamino)-2-oxo-2H-1-benzopyran-3-yl]benzoic acid in 200 mL THF, stir at room temperature for 1 h, add a solution of 2.27 g sodium borohydride in 4.8 mL water over 30 min, stir for 1 h, acidify with acetic acid, evaporate the THF under reduced pressure, add chloroform and water to the residue. Remove the organic layer and wash it with water, dry over anhydrous magnesium sulfate, evaporate to dryness, chromatograph on silica gel with chloroform, recrystallize from ethyl acetate/hexane to give 7-(diethylamino)-3-[4-(hydroxymethyl)phenyl]-2H-1-benzopyran-2-one as yellow needles (mp 153-154°). Stir 3.23 g 7-(diethylamino)-3-[4-(hydroxymethyl)phenyl]-2H-1-benzopyran-2-one and 86 mL phosphorus tribromide at 40-50° for 3 days, pour into ice-water, filter. Dissolve the solid in chloroform and wash with saturated aqueous sodium bicarbonate, wash with water, dry over anhydrous magnesium sulfate, evaporate to dryness, recrystallize from ethyl acetate/hexane to give 3-[4-(bromomethyl)phenyl]-7-(diethylamino)-2H-1-benzopyran-2-one as yellow needles (mp 166-167°).)

HPLC VARIABLES
Column: 250 × 4.6 Inertsil C8
Mobile phase: Gradient. A was MeOH:20 mM pH 7.5 Tris-acetate buffer 78:22. B was MeOH:20 mM pH 7.5 Tris-acetate buffer 90:10. A:B 100:0 for 20 min, to 65:35 over 30 min, to 35:65 (step gradient), to 10:90 over 20 min.
Flow rate: 1
Injection volume: 5
Detector: F ex 400 em 475

CHROMATOGRAM
Retention time: 52
Internal standard: 3α,7α,12α-trihydroxy-26a,26b-dihomo-27-nor-5β-cholestan-26b-oic acid (60)
Limit of detection: 15 fmole

OTHER SUBSTANCES
Simultaneous: numerous other bile acids, cholic acid

KEY WORDS
derivatization

REFERENCE
Kurosawa,T.; Sato,H.; Sato,M.; Takechi,H.; Machida,M.; Tohma,M. Analysis of stereoisomeric C_{27}-bile acids by high-performance liquid chromatography with fluorescence detection, *J.Pharm.Biomed.Anal.*, **1997**, *15*, 1375–1382.

SAMPLE
Matrix: urine
Sample preparation: Centrifuge urine, pass 40 mL urine through a pre-washed C18 Sep-Pak SPE cartridge, wash with 10 mL water, elute with 10 mL MeOH. Evaporate to dryness and take up the residue in 10 mL 100 mM pH 5.0 sodium acetate buffer, add 100 μg β-glucuronidase, add 100 μg cholylglycine hydrolase, heat at 37° for 36 h, pass the mixture through a pre-washed C18 Sep-Pak SPE cartridge, wash with 10 mL water, elute with 10 mL MeOH. Evaporate to dryness and take up the residue in 1 mL MeOH, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 37-50 μm Corasil C18
Column: 100 × 85 μm μBondapak C18 radial pack
Mobile phase: MeCN:MeOH:water:acetic acid 70:20:70:1
Flow rate: 2
Injection volume: 50
Detector: RI

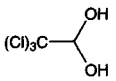
CHROMATOGRAM
Retention time: 32
Limit of detection: 1000 ng

OTHER SUBSTANCES
Extracted: ursodiol, bile acids, deoxycholic acid

KEY WORDS
SPE

REFERENCE
Batta,A.K.; Shefer,S.; Batta,M.; Salen,G. Effect of chenodeoxycholic acid on biliary and urinary bile acids and bile alcohols in cerebrotendinous xanthomatosis; monitoring by high performance liquid chromatography, *J.Lipid Res.*, **1985**, *26*, 690–698.

Chloral hydrate



Molecular formula: $C_2H_3Cl_3O_2$
Molecular weight: 165.40
CAS Registry No.: 302-17-0
Merck Index: 2113

SAMPLE

Matrix: blood
Sample preparation: 250 µL Plasma + 250 µL 150 µg/mL 4-chloro-1-butanol in water, vortex, add 20 µL benzoyl chloride dropwise on the surface, vortex, add 250 µL 4 M NaOH, rotate at medium sped for 10 min, add 10 mL pentane, rotate for 5-7 min, centrifuge at 2000 g for 5 min. Remove the organic layer and evaporate it to dryness at 45-50°, reconstitute the residue in 100 µL MeOH, inject a 5-10 µL aliquot.

HPLC VARIABLES

Guard column: 7 µm RP-18 (Brownlee)
Column: 150 × 4.6 5 µm Ultrasphere ODS
Mobile phase: MeCN:MeOH:water 30:30:40
Flow rate: 2
Injection volume: 5-10
Detector: UV 237

CHROMATOGRAM

Retention time: 12 (for trichloroethanol, the active metabolite)
Internal standard: 4-chloro-1-butanol (7)
Limit of quantitation: 7000 ng/mL

OTHER SUBSTANCES

Noninterfering: acetaminophen, salicylic acid, barbiturates

KEY WORDS

plasma; derivatization

REFERENCE

Gupta,R.N. Determination of trichloroethanol, the active metabolite of chloral hydrate, in plasma by liquid chromatography, *J.Chromatogr.*, **1990**, *500*, 655–659.

SAMPLE

Matrix: microsomal incubations
Sample preparation: 1 mL Microsomal incubation + 200 µL 3 mg/mL 2,4-dinitrophenylhydrazine in 2 M HCl (freshly prepared), shake for 10 min, extract twice with 1 mL portions of ethyl acetate. Combine the extracts and evaporate them to dryness under reduced pressure, reconstitute with 500 µL MeCN, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 µm Hypersil ODS
Mobile phase: MeCN:water 55:45
Flow rate: 2
Detector: UV 330

CHROMATOGRAM

Retention time: 11.1

OTHER SUBSTANCES

Extracted: acetaldehyde, acetone, 9-chloroanthracene, formaldehyde, malondialdehyde, propionaldehyde

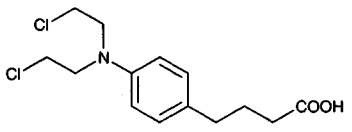
KEY WORDS

mouse; liver; derivatization

REFERENCE

Ni,Y.-C.; Wong.T.-Y.,; Lloyd,R.V.; Heinze,T.M.; Shelton,S.; Casciano,D.; Kadlubar,F.F.; Fu,P.P. Mouse liver microsomal metabolism of chloral hydrate, trichloroacetic acid, and trichloroethanol leading to induction of lipid peroxidation via a free radical mechanism, *Drug Metab.Dispos.*, **1996**, *24*, 81–90.

Chlorambucil



Molecular formula: $C_{14}H_{19}Cl_2NO_2$
Molecular weight: 304.22
CAS Registry No.: 305-03-3
Merck Index: 2116

SAMPLE

Matrix: blood
Sample preparation: Directly inject a 20-30 μL aliquot of plasma.

HPLC VARIABLES

Guard column: 50 × 3.9 30-32 μm pellicular Co:Pell ODS
Column: 250 × 4.6 10 μm PXS-10/25 ODS (A) or 150 × 4.6 5 μm Chromegabond MC-18 (B)
Mobile phase: MeOH:20 mM KH_2PO_4 50:50 (A) or 55:45 (B)
Flow rate: 1.5 (A), 1.0 (B)
Injection volume: 20-30
Detector: UV 254, UV 280, F ex 285 em 320 (cut-off filter)

CHROMATOGRAM

Retention time: 15 (A), 9 (B)
Limit of detection: 11.9 pmole

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

plasma; direct injection

REFERENCE

Zakaria,M.; Brown,P.R. Rapid assay for plasma chlorambucil and phenyl acetic mustard using reversed-phase liquid chromatography, *J.Chromatogr.*, **1982**, *230*, 381–389.

SAMPLE

Matrix: blood
Sample preparation: Add 1 mL 2 μg/mL IS in MeOH to 100 μL plasma, mix, centrifuge at -20° at 4500 g for 15 min. Remove the supernatant and dry it under vacuum. Resuspend the residue in mobile phase, centrifuge at 4° at 200 g for 5 min, inject a 10 μL aliquot.

HPLC VARIABLES

Guard column: μBondapak C18 Guard-Pak
Column: 100 × 8 4 μm Nova-Pak C18 Radial-Pak
Mobile phase: MeOH:water:100 mM tetrabutylammonium hydroxide in water 70:25:5, adjusted to pH 7.4 with orthophosphoric acid
Flow rate: 3
Injection volume: 10
Detector: U 254, UV 280

CHROMATOGRAM

Retention time: 3.5
Internal standard: β mono(trifluoromethyl) analogue (4.8)
Limit of detection: 60 ng/mL

KEY WORDS

mouse; plasma

REFERENCE

Workman,P.; Oppitz,M.; Donaldson,J.; Lee,F.Y. High-performance liquid chromatography of chlorambucil analogues, *J.Chromatogr.*, **1987**, *422*, 315–321.

SAMPLE

Matrix: blood

Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18

Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)

Column temperature: 30

Flow rate: 0.8

Injection volume: 50

Detector: UV 259

CHROMATOGRAM

Retention time: 10.00

Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen;

tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, tissue

Sample preparation: Blood. Mix 250 μL blood with 2 volumes of cold MeCN:DMSO 95:5. Centrifuge, dilute with 2 volumes of mobile phase, inject a 20 μL aliquot. Tissue. Homogenize tissue with 2 volumes of cold MeCN. Centrifuge the homogenate, dilute with 2 volumes of mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: Hypersil ODS-2 C18
Column: 100 × 4.0 Hypersil ODS-2 C18
Mobile phase: MeCN:acetic acid:water 72.5:0.5:27
Flow rate: 0.6
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 2.5
Limit of detection: 90 ng/mL

KEY WORDS

lung; liver; kidney; human; dog; rat; rabbit; pharmacokinetics

REFERENCE

Saah,F.; Wu,W.-M.; Eberst,K.; Marvanyos,E.; Bodor,N. Design, synthesis, and pharmacokinetic evaluation of a chemical delivery system for drug targeting to lung tissue, *J.Pharm.Sci.*, **1996**, *85*, 496–504.

SAMPLE

Matrix: blood, tissue

Sample preparation: Plasma. Plasma + 100 μL phenylpropionic mustard and chlorambucil propyl ester in MeOH + 3 mL MeCN + 1 mL dichloromethane + 2 mL hexane, shake, centrifuge at 2000 g at 4° for 10 min. Remove the top two organic layers and evaporate them to dryness, reconstitute the residue in 200 μL MeOH:hexane 3:1, inject an aliquot. Tissue. Tissue + 5 mL chilled MeCN + 100 μL phenylpropionic mustard and chlorambucil propyl ester in MeOH, sonicate for 30 s on ice, shake vigorously, centrifuge at 2000 g at 4° for 10 min. Remove the top organic layer and evaporate it to dryness, reconstitute the residue in 200 μL MeOH:hexane 3:1, inject an aliquot. (Final concentrations of phenylpropionic mustard should be 2.5 μg/mL and 0.25 μg/g and of chlorambucil propyl ester should be 2.5 μg/mL and 2.5 μg/g in plasma and tissue respectively.)

HPLC VARIABLES

Guard column: pellicular C18 (Waters)
Column: 100 × 4.6 5 μm RAC III Partisil 5 ODS 3
Mobile phase: Gradient. A was water:acetic acid 98:2. B was MeCN:acetic acid 98:2. A:B from 30:70 to 65:35 (sic) over 17 min (Waters curve no. 7), maintain at 65:35 for 7 min

Flow rate: from 1.5 to 2.5 over 17 min, maintain at 2.5 for 7 min
Detector: UV 254

CHROMATOGRAM
Retention time: 13.7
Internal standard: phenylpropionic mustard (12.6), chlorambucil propyl ester (19.0)
Limit of detection: 25 ng/mL

OTHER SUBSTANCES
Extracted: phenylacetic mustard, chlorambucil tert-butyl ester

KEY WORDS
plasma; pharmacokinetics; rat; brain

REFERENCE
Greig,N.H.; Stahle,P.L.; Shetty,H.U.; Genka,S.; John,V.; Soncrant,T.T.; Rapoport,S.I. High-performance liquid chromatographic analysis of chlorambucil tert.-butyl ester and its active metabolites chlorambucil and phenylacetic mustard in plasma and tissue, *J.Chromatogr.*, **1990**, *534*, 279–286.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 201.7

CHROMATOGRAM
Retention time: 22.387

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: microsomal incubations

Sample preparation: Add microsomal incubation to 2 volumes methyl ethyl ketone, vortex for 45 s, centrifuge at 100 g for 5 min. Remove the organic layer and evaporate it to dryness under vacuum, reconstitute the residue in 1 mL MeOH, filter (0.2 μm nylon), inject an aliquot.

HPLC VARIABLES

Column: 150 × 2 4 μm Nova Pak C18
Mobile phase: MeOH:0.05% trifuoroacetic acid, pH 2.25 52:48
Flow rate: 0.35
Detector: UV 258 for 15 min then UV 249

CHROMATOGRAM

Retention time: 32
Internal standard: 3-(4-hydroxyphenyl)propionic acid (8)
Limit of quantitation: 40 ng

OTHER SUBSTANCES

Extracted: chlorambucil N-oxide

KEY WORDS

rat; liver

REFERENCE

Chandler,K.J.; McCabe,J.B.; Kirkpatrick,D.L. High-performance liquid chromatographic method for the separation of chlorambucil and its N-oxide prodrug, *J.Chromatogr.B*, **1994**, *652*, 195–202.

SAMPLE

Matrix: reaction mixtures
Sample preparation: Mix an aliquot with an equal volume of 20 mM pH 4.4 KH_2PO_4, centrifuge, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Microsorb C8
Mobile phase: MeOH:20 mM pH 4.4 KH_2PO_4 65:35
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 13.2
Limit of detection: 750 ng/mL

REFERENCE

Lunn,G.; Sansone,E.B.; Andrews,A.W.; Hellwig,L.C. Degradation and disposal of some antineoplastic drugs, *J.Pharm.Sci.*, **1989**, *78*, 652–659.